



EXPERIMENTAL/ORIGINAL PAPER

Echocardiographic assessment of left ventricular midwall mechanics in spontaneously hypertensive rats

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KEYWORDS

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Abstract Aims The present study was attempted to determine whether LV mid-wall mechanics yielded different conclusions about LV systolic function than the assessment of endocardial LV mechanics by echocardiography in spontaneously hypertensive rats (SHR).

Methods and results Thirty-six (18 Wistar normotensive (W), 18 [SHR]) anesthetized rats were studied with two-dimensional directed M-mode echocardiogram to analyze LV structure (LV diameter, left ventricular wall thickness and LV mass [LVM]) and LV function (endocardial shortening [ES] and midwall shortening [MS]). Measurements were made from three consecutive cardiac cycles on the M-mode tracings.

There was no significant difference in LV dimension. LVM was higher in SHR (SHR: 595 ± 111 mg, W: 413 ± 83 mg— $p < 0.01$). ES was higher in SHR (SHR: $64.1 \pm 6\%$, w: $58.2 \pm 4\%$ — $p < 0.01$), whereas no significant difference was found in MS (SHR: $27 \pm 4\%$, W: $27.6 \pm 4\%$ —ns). Twelve of 18 (66%) SHR showed endocardial shortening higher than normally predicted, compared with 3/18 (16%) with observed enhanced MS ($p < 0.01$).

Conclusion These results suggest that the analysis of midwall mechanics by echo allows us to better understand the LV performance in SHR and that the exaggerated endocardial motion could not represent a really *supernormal* systolic performance. © 2003 Published by Elsevier Ltd on behalf of The European Society of Cardiology.

Introduction

Studies on rat have provided basic knowledge about the pathophysiology of arterial hypertension, particularly about left ventricle hypertrophy and function.^{1–3} Nevertheless, the assessment of

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left ventricle (LV) hypertrophy in rat has required animal sacrifice, making it impossible to study in vivo geometry or function under different conditions. The development of non-invasive techniques to accurately and repeatedly evaluate ventricular morphology and systolic function would provide a means for *measuring* changes in heart geometry and/or function with preservation of animal life.

Echocardiography, widely recognized to provide the most accurate quantitative non-invasive measurement of LV morphology and function in humans,^{4–6} has taken a great interest in assessing LV geometry and function in small animals such as rats, hamster and mice during the last decade.^{7–11}

Supernormal LV performance has been recognized in subsets of humans^{12,13} and animals with experimental hypertension,¹⁴ due to an increased contractility and/or the use of preload reserve, especially in the absence of adequate LV hypertrophy.^{15,16} As shown in human studies,^{17,18} in rats with renovascular hypertension¹⁹ and in Dahl salt-sensitive animals,²⁰ the assessment of LV function by measuring the extent of fiber shortening at the endocardium level is less appropriate than using the value calculated at the midwall. This region represents both the anatomical site of circumferential myocardial fibers and the approximate level where mean transmural wall stress is applied. Since LV systolic function has been reported to be *supernormal* on the basis of endocardial fractional shortening in rats with renovascular hypertension, this finding might be produced by the same mismatch seen in humans.¹³ Despite the widespread use of the spontaneously hypertensive rats (SHR) as a model of experimental hypertension, there is no available information of in vivo midwall LV performance in this rat strain.

Accordingly, the present study was attempted to determine whether LV midwall mechanics yielded different conclusions about LV systolic function than the assessment of endocardial LV mechanics by echocardiography in SHR.

Material and methods

Animals

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No 85-23, revised 1966) with 18 SHR and 18 normotensive Wistar (W) rats (16–18 weeks old). From the age of 3 months, systolic blood pressure (SBP) was weekly determined by

the tail-cuff method in each rat. All animals were allowed to feed ad libitum and had free access to tap drinking water.

Echocardiographic evaluation

Rats were lightly anesthetized (35 mg/kg pentobarbital sodium, i.p.) and placed prone on a specially designed apparatus with their chest shaved. Two-dimensionally targeted M-mode tracings were recorded through the anterior and posterior LV walls, at a paper speed of 50 mm/s during 10 cycles with a commercially available echocardiographic machine equipped with a 7.5 MHz oscillating single-crystal mechanical transducer (Interspec XL-3-Ambler, PA). Long-axis images of the aorta, left atrium and LV were first obtained; rotation 90° from the long-axis view produced a short axis view of the heart. LV images in this view were recorded at the level of the midventricle with a ventricular image as small and round as possible. The M-mode cursor was positioned using two-dimensional guidance in this place to obtain images of the LV so as to maximize ventricular diameter and minimize wall thickness from this transducer position (Fig. 1).

Determination of echocardiographic indexes

Measurements were made using criteria analogous to those recommended by the American Society of Echocardiography for use in humans²¹ from at least three consecutive cardiac cycles on the M-mode tracings. All tracings were analyzed with an off-line analysis system (Scion Image).

LV mass (LVM) was calculated according to the method of Devereux and Reichek,²² and LVM index (LVMI) was calculated by normalizing LVM for body weight (BW). Relative wall thickness (RWT), endocardial shortening (ES) and midwall shortening (MS) (Fig. 2) was measured as had been described in previous reports.^{17,21} Meridional end-systolic stress (ESS) was calculated using SBP values, measured in the awoken animals within 48 h before the echocardiogram, according to Douglas et al.²³

Interpretative variability

All measurements were analyzed in 11 randomly selected animals by two observers (E.E., A.T.). Intraobserver and interobserver differences were calculated as the difference between two observations divided by the mean of the observations,²⁴ by the limits of agreement defined as the mean difference between the observations ($\pm 2D$)²⁵ and

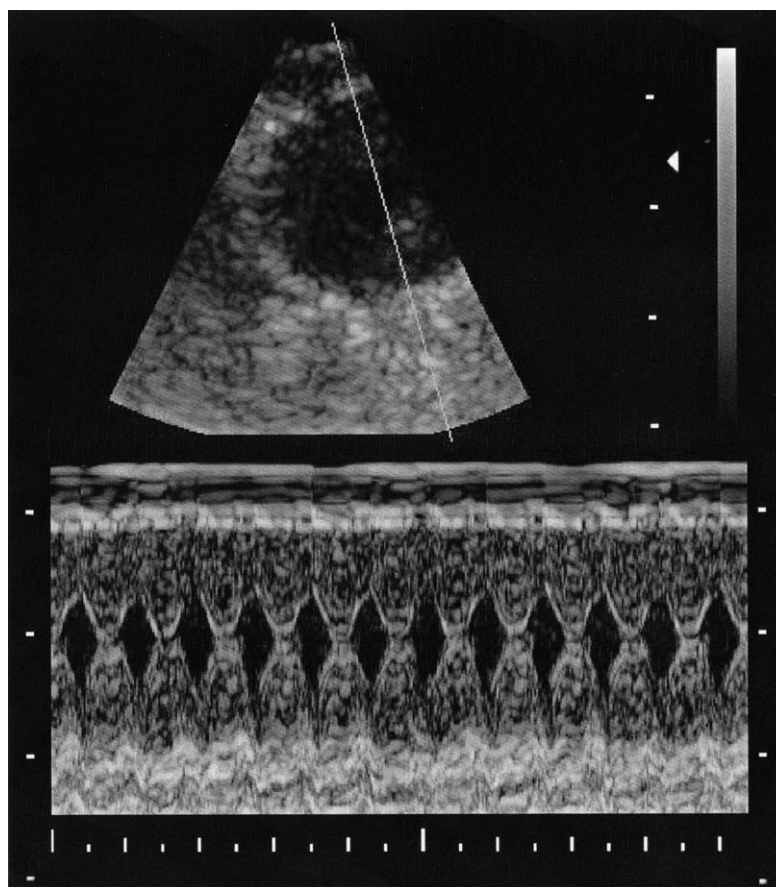


Figure 1 A transthoracic two-dimensional directed M-mode echocardiogram with a short axis view of circular midventricle from a W rat weighing 280 g at the age of 16 weeks. Each point in dimension scale is 0.5 cm and 100 ms in time scale. Heart rate is 350 bpm.

by the correlation coefficient for all ventricular dimensions.²⁶

Pathologic studies

After the echocardiographic study, animals were euthanized under ether anesthesia. The hearts were removed and trimmed off pericardium, fat and blood vessels. The atria and the right ventricular free wall were carefully removed; and the LV was blotted and weighed on an analytical balance.

Statistical analysis

Results are expressed as mean values \pm SD. Student's *t*-test or one-way ANOVA were used to compare differences with Student–Newman–Keuls as post hoc test, as appropriate. χ^2 statistics were used to compare categorical variables between groups. Correlation coefficient was determined with the least square method, and the Bland–Altman plot was used to describe the comparison between LV-weight and echocardiographically

determined LV mass. Value of $p < 0.05$ was considered to indicate significant differences.

Results

Interpretative variability

The intraobserver and interobserver variabilities for all measurements were 6.8% and 14%, respectively. The mean of the differences between observations for intra-interobserver determinations was 0.02 ± 0.016 cm and 0.04 ± 0.03 cm, respectively. Intraobserver correlation for ventricular dimension measurements was 0.98 for all dimensions; and interobserver correlation was 0.95.

LV structure

Table 1 shows data about BW, SBP and LV anatomy in SHR and W animals. There was no significant difference in echo-determined LV chamber dimension

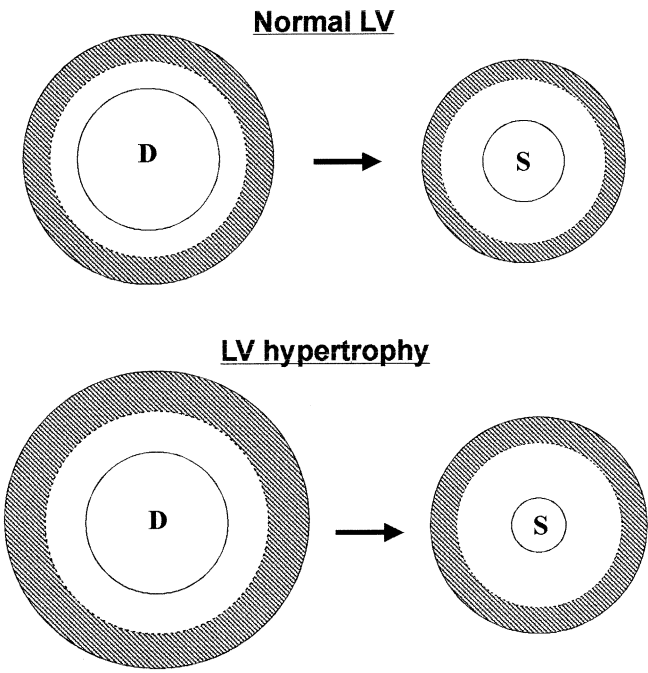


Figure 2 Two-shell geometry of left ventricle explained by schematic cross-section of left ventricle. A hypertrophied LV ventricle (LV hypertrophy) thickness of the inner shell is greater than the normal LV. At end-systole (S), the thickness of the inner shell is greater than that of the outer shell, indicating that end-diastolic (D) midwall fiber migrates in an epicardial direction in systole. This migration is more evident in LV hypertrophy.

between both groups of rats but LV systolic dimension appeared slightly increased in W animals.

LVM was higher in hypertensive than in normotensive rats, as were septal or anterior wall, posterior wall and relative wall thickness.

There was good linear correlation ($r = 0.84$; $p < 0.001$) with measured left ventricular weight as shown by the Bland–Altman plot in Fig. 3. The relationship between both parameters was best

fitted by a regression line of the form $y = 1.08X + 159.05$. The high values of intercept and mean of difference were probably due to the relative low frame rate of two-dimensional imaging, making true end-diastolic frame difficult to isolate.

LV systolic function

ES was higher in SHR ($64.1 \pm 6\%$) than in W ($58.2 \pm 6\%$ — $p < 0.01$) (Fig. 4A), whereas no significant difference was found in MS ($27 \pm 4\%$ in SHR vs. $27.6 \pm 4\%$ in W rats) (Fig 4B).

SBP was higher in SHR than in W as shown in Table 1; however, meridional end-systolic wall stress was lower in hypertensive rats (SHR 12 ± 3.3 kdyn/cm², W: 14 ± 4.2 kdyn/cm²— $p < 0.01$) (Fig. 5).

Distributions of endocardial shortening and mid-wall shortening data were examined, and the 5th and 95th percentiles were regarded as normal limits of LV performance. Therefore values below the 5th percentile were considered systolic left ventricular dysfunction and values above the 95th percentile hypercontractile state.

Twelve out of 18 SHR (66%) exhibited values of endocardial shortening higher than the normal

Table 1 Body weight, blood pressure and LV anatomy of Wistar and SHR rats			
	Wistar (n = 18)	SHR (n = 18)	p
BW (g)	265 ± 38	242 ± 22	<0.02
SBP (mmHg)	121 ± 14	179 ± 5	<0.001
LVM (mg)	413 ± 83	595 ± 111	<0.01
LVMI (mg/g)	1.57 ± 0.26	2.45 ± 0.36	<0.01
LVDd (cm)	0.50 ± 0.06	0.51 ± 0.05	n.s.
LVDs (cm)	0.20 ± 0.04	0.18 ± 0.03	n.s.
RWT	0.60 ± 0.10	0.72 ± 0.08	<0.01
AWdt (cm)	0.15 ± 0.01	0.19 ± 0.02	<0.001
PWdt (cm)	0.15 ± 0.02	0.18 ± 0.01	<0.001

BW: body weight, LVM: left ventricular mass, LVMI: left ventricular mass index, LVDd: left ventricular end-diastolic dimension, LVDs: left ventricular end-systolic dimension, RWT: relative wall thickness, AWd: anterior wall diastolic thickness, PWd: posterior wall diastolic thickness.

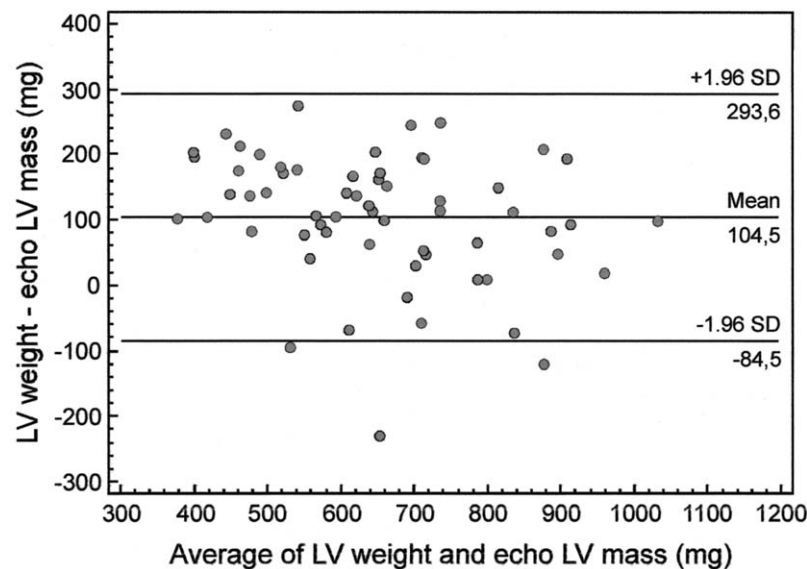


Figure 3 Bland–Altman plot showing the relation between echocardiographically and directly determined LVM from the 36 rats.

95th percentile, whereas three out of 18 (16%) showed enhanced midwall shortening ($p < 0.01$). Neither endocardial nor midwall shortening indicated depressed LV performance in SHR.

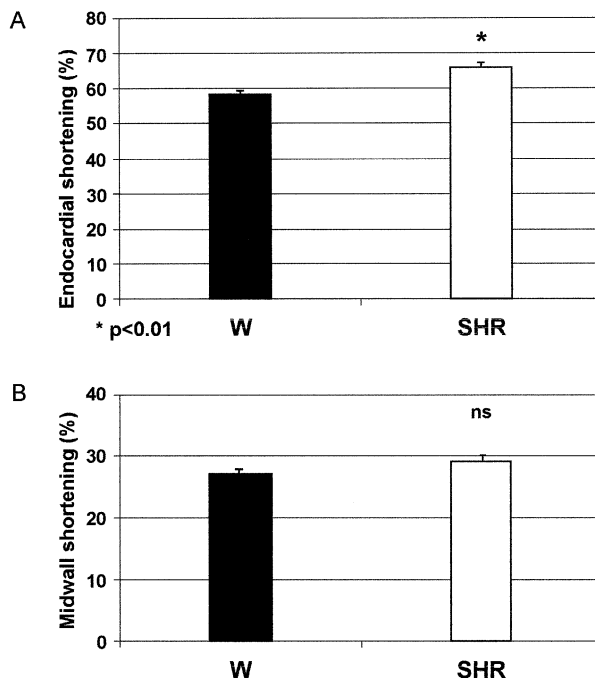


Figure 4 Bar graphs showing LV function in two groups of rats (W and SHR). The upper graphs (A) showed groups' means (\pm SD) for endocardial shortening for Wistar rats (solid bar) in relation to means for SHR rats (open bar). (B) The comparison of means (\pm SD) for midwall shortening between W (solid bar) and SHR (open bar) groups is shown. * $p < 0.01$.

Discussion

Determination of LV midwall mechanics in humans has been demonstrated to be a physiologically appropriate method for studying LV function without the potential artifact introduced by estimation of LV muscle shortening for the endocardial surface.^{18,27} De Simone et al.¹⁸ have shown that endocardial definition of LV shortening overestimates LV performance in patients with arterial hypertension and abnormalities of LV geometry, and also in Goldblatt rats on normal salt diet.¹⁹ Ono et al.²⁰ have recently shown the same overestimation in male Dahl-S salt-sensitive (Dahl S) rats. As in accordance with these observations, using endocardial shortening of LV minor axis yielded higher estimates of LV function in SHR than did the assessment of midwall shortening in the present study. In SHR rats, the number of animals exhibiting supernormal LV performance measured at the midwall was higher (16%) than that found in hypertensive humans (5%) and in Goldblatt rats (7.5%). In the other way, 66% of our hypertensive rats showed supernormal values of endocardial shortening, whereas 22% and 28% were reported in humans and Goldblatt rats, respectively. No animal in the present study exhibited systolic dysfunction, even when midwall LV mechanics were assessed, similar to that reported by De Simone et al.¹⁴ in hypertensive Goldblatt animals.

Echocardiographically derived ejection-phase indexes as well as endocardial fractional shortening are sensitive to loading conditions and, therefore, are incapable of accurately assessing

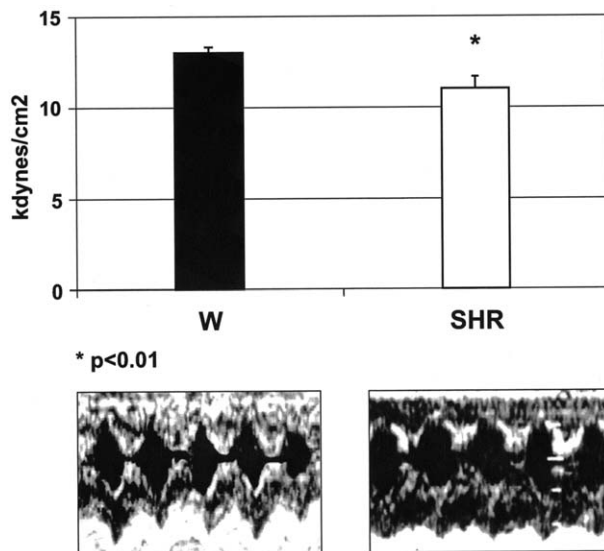


Figure 5 Upper: bar graphs of end-systolic stress of LV for both groups (W: solid bar; SHR: open bar). Bottom: M-mode echocardiographic tracings for normotensive (left) and hypertensive (right) rats. The two-dimensionally guided M-mode LV images were obtained at the level of papillary muscles.

alterations in intrinsic myocardial contractility.²³ Thus, it must be analyzed in relation to the forces that resist systolic emptying of the LV, i.e., the afterload.

End-systolic stress is a more appropriate measurement of afterload than either peak systolic pressure or peripheral vascular resistance and is both easier to derive and conceptually more appealing than characteristic aortic impedance. In addition, as our results show, LV hypertrophy in the SHR decreases end-systolic stress due to its inappropriate level of hypertrophy²⁸ and may either mask intrinsic contractile dysfunction or mimic a hypercontractile state.

In this study, the overestimation of LV systolic function by LV endocardial fractional shortening observed in SHR could be at least partially explained by the associated decrease in LV end-systolic wall stress (afterload).^{18,20} This overestimation could be interpreted as underestimation when we analyzed LV function during LV hypertrophy regression.²⁹

LV midwall fractional shortening, in contrast, is slightly affected by LV concentric geometry and LV systolic wall stress.¹⁷ In this study, the effect of LV hypertrophy was minimized by taking midwall rather than endocardium as other authors reported.³⁰

Several potential limitations of this study should be acknowledged. First, peak aortic pressure measured within 48 h before the echo study by tail-cuff method was used as a surrogate for LV end-systolic

pressure. This is unlikely to have introduced a significant error, because Reichek et al.²⁷ reported good correlations between micro-manometer-determined LV pressure at end-ejection and both peak LV pressure ($r = 0.97$) and cuff systolic arterial pressure ($r = 0.89$). Second, meridional rather than circumferential wall stress was used; the latter requires, furthermore, a measurement of the long axis of the ventricle, which cannot be reliably determined with our method. Although circumferential stress is theoretically more appropriate because it occurs in the same plane as minor axis shortening,²³ several studies showed that both meridional and circumferential stresses are related to ventricular performance.^{30–33} Third, the use of a single M-mode echocardiographic dimension for stress and shortening determinations assumes symmetrical wall motion and precludes the application of this method in LV with regional wall motion abnormalities.

Finally, our anesthetic decreased heart rate to about 40% because resting heart rate in awoken rats is approximately 400 bpm. Thus, the values of echocardiographic indexes might not reflect the physiological conditions of the animals. Because the effects of anesthesia appear to be homogeneous among the studied rats, we may safely use these indexes for the interindividual comparisons.

Despite these potential limitations, our data suggest that echocardiographic indexes of LV systolic function may be used to characterize LV performance in SHR, a model very similar to human hypertension. Since these measurements are obtained non-invasively, serial determinations are possible and may be applied to a number of studies. The analysis of midwall mechanics by midwall shortening fraction will allow us to better understand the LV performance in hypertrophied LV, suggesting that the exaggerated endocardial motion in the hypertrophied heart could not represent a really supernormal systolic performance.

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